Electrochemical Detection of Biological Pathogens

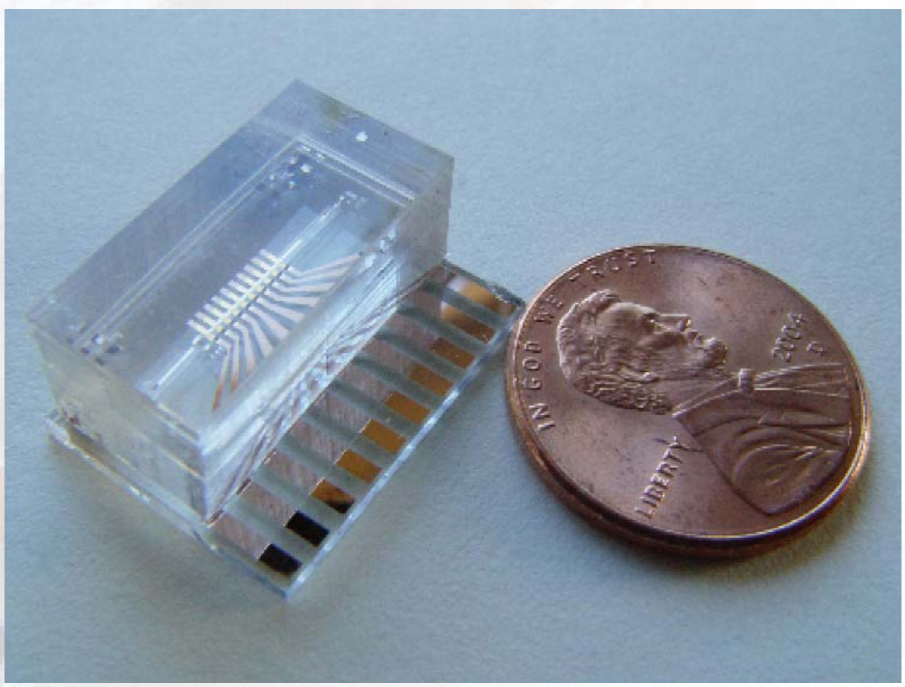
For more information contact **David Sopchak**
(925) 422-2240, sopchak1@llnl.gov

Detection techniques are essential for identification of DNA fragments for a variety of applications, including drug discovery, DNA sequencing, and pathogen detection. The most common techniques for DNA separations are performed with fluorescent labels using capillary electrophoresis to sequence the samples of interest. While this technique provides excellent sensitivity, it involves optics and lasers that are relatively expensive and difficult to miniaturize.

Electrochemical detection techniques offer the potential of high sensitivity, low cost, and simple integration, which would prove beneficial in the advancement of sequencing and DNA fragment analysis. The use of electrochemical detection has previously been investigated using redox active molecules in DNA hybridization studies, and for detectors in capillary gel electrophoresis. While these techniques have exhibited excellent sensitivity, they have not been applied to specific systems for applications to homeland security. This effort will look at the use of custom-patterned electrode materials, which can be directly integrated with capillary gel electrophoresis devices for evaluation against the more proven fluorescent detection techniques.

Project Goals

The goal of this project is to produce an electrochemical detector for a commercial (Beckman-Coulter) capillary electrophoresis device that will serve as a testbed for further refinement for smaller devices.



Photograph of prototype electrochemical detector: PDMS on glass with gold electrodes.

Relevance to LLNL Mission

The problem of pathogen detection is important for homeland security. Low cost, miniature detection systems will eventually provide new capability to the individual, or to first-response teams, for a variety of field scenarios. The particular problem addressed by this effort couples directly to systems currently being considered for these applications at LLNL.

FY2004 Accomplishments and Results

In FY2003 we produced the first of the prototype electrochemical detector devices (see figure). ACLARA Inc. provided three anthraquinone-based eTag™ samples for testing. In FY2004, electrochemical hardware (an existing Pine potentiostat, LabJack data acquisition device connected to a Mac) was set up, and software was written for data acquisition. A software workaround for removal of emf-related noise during data acquisition was implemented and was able to lower the apparent noise to ~30 nA.

Tests to establish behavior of gold, platinum, and glassy carbon electrodes towards hydroquinone in 1 M potassium chloride were conducted. (Hydroquinone resembles anthraquinone in its electrochemical behavior and has been well studied.) The glassy carbon electrodes displayed the best sensitivity and lowest background current, with detection limits of about 100 nM, using conventional techniques.

Electrochemical testing of anthraquinone in aqueous (1 M KCl) solutions followed, in preparation for using anthraquinone-modified eTags™. We found that the redox potential of anthraquinone was beyond the cathodic breakdown potential for the solvent, rendering detection of these eTags™ impossible in aqueous systems.

Testing of the electrochemical detection device that was fabricated in FY2003 was also performed. Detection limits were found to be sub-picomole, limited only by the background noise of the potentiostat being used. Unfortunately, the gold microelectrodes used were not inert enough to withstand the potential at which they were poised, and dissolved completely after an hour's use.

A picoampere-range potentiostat from Bioanalytical Systems was purchased to improve detection limits. To address both the electrode robustness issue and to lower the detection limits, we are fabricating electrodes made from CVD boron-doped diamond films. Boron-doped diamond has been shown to have outstanding robustness and excellent sensor properties, including low background current, in electrochemical systems. We believe with these new electrodes and equipment, we can push detection limits to the attomole range and below, which is the state of the art for electrochemical detection.

Related References

1. Woolley, A., K. Lao, A. Glazer, and R. Mathies, "Capillary Electrophoresis Chips with Integrated Electrochemical Detection," *Analytical Chemistry,* **70,** pp. 684-688, 1998.
2. Backofen, U., F. Matysik, and C. Lunte, "A Chip-Based Electrophoresis System with Electrochemical Detection and Hydrodynamic Injection," *Analytical Chemistry,* **74,** pp. 4054-4059, 2002.
3. Sopchak, D., B. Miller, R. Kalish, Y. Avvigal, and X. Shi, "Dopamine and Ascorbate Analysis at Hydrodynamic Electrodes of Boron-Doped Diamond and Nitrogen Incorporated Tetrahedral Amorphous Carbon," *Electroanalysis,* **14,** (7-8), pp. 473-478, 2002.
4. Sopchak, D., B. Miller, Y. Avigal, and R. Kalish, "Rotating Ring–Disk Electrode Studies of the Oxidation of P-Methoxyphenol and Hydroquinone at Boron-Doped Diamond Electrodes," *Journal of Electroanalytical Chemistry,* **538-539,** pp. 39-45, 2002.